Serum lipid profile and fatty acid composition of erythrocyte phospholipids in children and adolescents with primary hyperlipidemia

Serum lipid profile and fatty acid composition of erythrocyte phospholipids in children and adolescents with primary hyperlipidemia

Journal:	*International Journal of Food Sciences and Nutrition*
Manuscript ID	CIJF-2016-0721.R1
Manuscript Type:	Research Paper
Date Submitted by the Author:	n/a
Complete List of Authors:	Deon, Valeria; Università degli Studi di Milano, Department of Food, Environmental and Nutritional Science; Division of Human Nutrition Del Bò, Cristian; Università degli Studi di Milano, Department of Food, Environmental and Nutritional Sciences; Division of Human Nutrition Guaraldi, Federica; Universita degli Studi di Torino, Department of Health, Sciences and Pediatric Gargari, Giorgio; Università degli Studi di Milano, Department of Food, Environmental and Nutritional Science; Division of Food Microbiology and Bioprocessing Bosusco, Andrea; Universita degli Studi di Torino, Department of Health, Sciences and Pediatric Simonetti, Paolo; Università degli Studi di Milano, Department of Food, Environmental and Nutritional Science; Division of Human Nutrition Riso, Patrizia; Università degli Studi di Milano, Department of Food, Environmental and Nutritional Science; Division of Human Nutrition Guardamagna, Ornella; Universita degli Studi di Torino, Department of Health, Sciences and Pediatric
Keywords:	Children, primary hyperlipidemia, erythrocytes, phospholipids, lipid profile, omega-3 index

Serum lipid profile and fatty acid composition of erythrocyte phospholipids in children and adolescents with primary hyperlipidemia

Valeria Deon[1], Cristian Del Bo'[1], Federica Guaraldi[2], Giorgio Gargari[3], Andrea Bosusco[2], Paolo Simonetti[1], Patrizia Riso[1]*, Ornella Guardamagna[2]

[1]*Department of Food, Environmental and Nutritional Sciences, Division of Human Nutrition, Università degli Studi di Milano, Milan, Italy*

[2]*Department of Health, Sciences and Pediatric, Università degli Studi di Torino, Turin, Italy*

[3]*Department of Food Environmental and Nutritional Sciences, Division of Food Microbiology and Bioprocessing, Università degli Studi di Milano, Milan, Italy*

***Corresponding author:**

Prof. Patrizia Riso, PhD

Department of Food, Environmental and Nutritional Sciences, Division of Human Nutrition

Università degli Studi di Milano

Via G. Celoria 2, 20133 Milan, Italy

E-mail: patrizia.riso@unimi.it; Phone: +39 0250316726; Fax: +39 0250316721

Word count: 6246

Keywords: Children; primary hyperlipidemia; erythrocytes; phospholipids; lipid profile; omega-3 index.

ABSTRACT

This study aimed at characterising the fatty acid (FA) composition of red blood cell (RBC) phospholipids in children and adolescents with primary hyperlipidemia, and to ascertain potential association with serum lipid profile and dietary factors. At this purpose, 54 probands aged 6-17 years were recruited. Subjects showed a low omega-3 index (eicosapentaenoic acid, EPA + docosahexaenoic acid, DHA<4%). Compared to males, females had a trend towards lower levels of total monounsaturated fatty acids (MUFA) and MUFA/saturated fatty acids (SFAs) ratio in RBCs. An inverse relationship between MUFA concentration in RBCs and serum cholesterol or HDL-C/triglycerides ratio was found. Omega-6 polyunsaturated fatty acids (n-6 PUFA) were positively associated to serum HDL-C levels, and inversely to dietary cholesterol. Fibre intake was positively associated with MUFA/SFA ratio. In conclusion, we provide the first experimental data on phospholipid FA composition of RBCs in hyperlipidemic children, showing sex differences and an overall low omega 3-index.

Introduction

Hyperlipidemias are disorders of lipoprotein metabolism characterized by an increase in serum total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), or triglycerides (TG). These disorders, both primary (monogenic and polygenic inherited forms) and secondary (related to other diseases) forms, are considered a major cause of atherosclerosis and cardiovascular disease (CVD) (Newman et al. 1986; Ahmed et al. 1998; Groner et al. 2006; Haney et al. 2007).

The atherosclerotic process begins early in life, thus children and adolescents affected by primary hyperlipidemia show a particularly high risk to develop CVD later in life (Durrington 2003), which is mainly secondary to chronic exposure to elevated LDL-C, accumulating in the intima-media of large muscular arteries (Durrington 2003; McGill et al. 2000). Healthy lifestyle, which includes appropriate dietary pattern in agreement with the expert panel guidelines for CV health and risk reduction in children and adolescents (Expert panel …, 2011), physical activity and weight loss in case of excessive body weight, is the cornerstone in the treatment of hyperlipidemia and represents an important target for CVD prevention (Haney et al. 2007; Catapano et al. 2011). It has been suggested that the lipid composition of RBC membranes can be considered as an additional risk factor in the progression of atherosclerosis and coronary heart disease (Lausada et al. 2007; Tziakas et al. 2010). The fatty acid (FA) composition of red blood cells (RBCs) generally reflects the last three months of dietary fat intake, and it is thought to be a biomarker of the tissue fatty acid status (Sarkkinen et al. 1994; Kuratko et al. 2009; Brigandi et al. 2015).

Since erythrocytes are incapable of de novo phospholipid synthesis, chain elongation or desaturation of fatty acids, the major pathway to renew the RBC phospholipids is the direct exchange from plasma lipoproteins to the erythrocyte (Marks et al. 1960;

Farquhar & Ahrens 1963; Reed 1968; Hodson et al. 2008). Thus, the RBC FA composition is postulated to better and earlier reflect the pathology of lipid metabolism, in respect to lipoprotein changes in blood serum, which are affected by recent food consumption (Sarkkinen et al. 1994; Harris & Von Schacky 2004; Novgorodtseva et al. 2011).

Studies conducted in adults found a correlation between altered FA composition in RBCs and coronary heart disease, arterial hypertension, dyslipidemia and other atherosclerosis-related diseases (Engelmann et al. 1992; Taylor 1994; Antoku et al. 2000; Lausada et al. 2007; Vayá et al. 2009; Ristic-Medic et al. 2009; Tziakas et al. 2010; Novgorodtseva et al. 2011; Jacobs et al. 2014). Harris & Von Schacky (2004) demonstrated that a low content of eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids in the RBC membranes is strongly associated with coronary hearth diseases. The total levels of EPA and DHA in RBCs, defined as omega-3 index, was suggested as an additional biomarker of CV risk, and a predictive parameter for morbidity and mortality from CVD (Harris & Von Schacky 2004). Since most of this class of compounds is contained in cell membranes, the index has been also calculated on RBC phospholipids and other cell types; moreover, comparison with data obtained in plasma has been performed (Harris & Thomas 2010; Rice et al. 2016).

Despite the potential impact of knowing the RBC membrane FA composition in patients affected by lipoprotein disorders in terms of risk prediction, studies are still limited and mainly performed in adults. In this regard, trials carried out in children were mainly aimed at characterizing the phospholipid FAs of RBCs in healthy subjects (Laryea et al. 1990; Jakobit et al. 2009; Cortés et al. 2012), or in patients affected by other diseases (Decsi et al. 2002; Vanderjagt et al. 2003; Trebbe et al. 2003; Chen et al. 2004; Bu et al. 2006; Vaisman et al. 2008; Burrows et al. 2011; Perona et al. 2013;

Brigandi et al. 2015; Bueno et al. 2015). Furthermore, data on gender difference in RBC composition in childhood and adolescence have not been adequately investigated.

The aim of the present study is to provide data on the RBC phospholipid FA composition in a population of Italian children and adolescents with primary hyperlipidemia, receiving medical nutrition therapy at least 6 months before the recruitment. Since RBC phospholipid FA composition is influenced by blood lipoprotein profile/concentration changes and diet we also investigated the association with serum biomarkers and dietary parameters.

Materials and methods

Subject enrolment and study design

Fifty-four children and adolescents with primary hyperlipidemia were recruited among pediatric patients cared at the Department of Health Science and Pediatrics of the University of Turin, after a screening for eligibility. Children and adolescents included in the study were 6-17 years old, affected by familial hypercholesterolemia (FH), familial combined hyperlipidemia (FCHL) or polygenic hypercholesterolemia (PHC). Exclusion criteria were: secondary dyslipidemia; obesity (body mass index (BMI) $\geq 90^{th}$ percentile, age and sex matched); renal, endocrine and liver disorders; or chronic diseases requiring drug treatment (i.e. immunologic, neurologic, or oncohematologic disorders). Participants were not on lipid-lowering treatments (including functional foods) in the previous 3 months and were not smoking. Children on diet therapy were selected after demonstrating appropriate compliance with dietary instructions, provided by a trained nutritionist, in the previous 2 months. Recruited subjects and their families were trained by a nutritionist to adhere to a properly dietary regimen, evaluated by a weekly food diary.

Diagnostic criteria of familial hyperlipidemia were based on accepted international standards (Guardamagna et al. 2011). FH was diagnosed in presence of LDL-C ≥ 95th percentile, parental LDL-C ≥ 190 mg/dL, tendon xanthomas and/ or cardiovascular disease (phenotype IIA). FCHL was diagnosed in children showing TC and/or TG >90th age- and sex-specific percentile, with at least one parent affected by hypercholesterolemia, hypertriglyceridemia, or both (IIA, IV, or IIB phenotype, respectively), with concomitant individual and familial lipid phenotype variability. Children with LDL-C levels >90th percentile and a family history of dominant inherited hypercholesterolemia, but not fulfilling the biochemical international diagnostic criteria of FH or FCHL were diagnosed with PHC.

All subjects enrolled underwent a medical examination in the morning between 8 and 10 a.m. on the day of recruitment. Physical parameters including height, weight and blood pressure were measured. Height and weight were measured to the nearest 0.1 cm and 0.1 kg respectively (Wunder SA.BI. S.r.l. Italy), with the patients wearing hospital gowns and had bare feet. BMI was calculated as body weight in kilograms divided by height squared in meters (kg/m^2). Systolic and diastolic blood pressure was measured with a mercury sphygmomanometer during the medical examination. Fasting blood samples were drawn by venipuncture for the analysis of the lipid profile and the FA composition of RBCs.

Children and their parents had received dietary recommendations based on the cardiovascular health integrated lifestyle diet (CHILD-1) for children with identified dyslipidemia, as in the American Academy of Pediatrics (Expert panel …, 2011). The essential features were: 55% of daily energy from carbohydrate, 15% from protein, 25-30% from total fat (saturated fat <10%, MUFA and PUFA 20% of total energy), dietary cholesterol <100 mg/1000 kcal and no more than 200 mg/day, and 10-25 g/day of

soluble fibre. In order to examine correct dietary habits, parents of the enrolled children, and probands themselves, were asked to fill in a weekly food diary to be provided at time of the visit. Detailed instructions on how to collect diet records were comprehensively explained by a nutritionist. The nutritional evaluation of macronutrients and FA content of the diet registered was performed with MètaDieta® Software using the Italian Food Composition Tables (Carnovale & Marletta 2000).

The study protocol complied with the principles of the Declaration of Helsinki, and was approved by the ethics committee of the City of Health and Science University Hospital of Turin (EC:CS377). The protocol and the purpose of the study were carefully explained to the participants and to their parents; a written informed consent was obtained from children's parents before enrolment in the study.

Blood sample collection and separation

Venous blood samples of 2.5 ml were drawn into vacutainer tubes containing lithium heparin (to obtain plasma and RBCs) or silicon (to obtain serum). Plasma and serum were separated by centrifugation at 1400 g for 15 min at 4°C. Plasma was stored at -80°C for further analysis, while serum was immediately analysed. The buffy layer of white blood cells was removed using a pipette, and RBCs were washed twice in an equal volume of a physiologic solution (0.9% NaCl, w/v). Two aliquots (0.5 ml) of RBCs were stored at -80°C until the analysis.

Serum lipid profile

Serum levels of TC, HDL-C and TG were directly determined by an automatic biochemical analyzer (Olympus AU2700, Japan), while the LDL-C concentration was

estimated using the Friedewald formula (LDL= TC-(HDL+TG/5). Non-high density lipoprotein cholesterol (non–HDL-C) was calculated as TC minus HDL-C.

Analysis of FA composition of RBC phospholipids

Extraction of RBC phospholipids was performed in accordance to the method previously described by Simonetti et al. (2002). Briefly, 1 ml of distilled water, 300 mg of RBC, 2.5 ml of butylated hydroxitoluene (110 µg/ml in methanol), and 5 ml of chloroform were transferred into 10 ml plastic tubes. The mixtures were shaken on vortex for 3 min and centrifuged at 1200 g x for 10 min. The chloroform phase was transferred into 10 ml plastic tubes. An aliquot of 2.5 ml chloroform was added to the tubes containing RBCs to perform the second extraction by vortexing for 3 min, and, after centrifugation at 1200 g x for 10 min, the supernatant was transferred into the corresponding tube. The chloroform phase was eluted on a silica cartridge (Sep-Pack PlusSilica, Waters), which was then evaporated under nitrogen steam. The silica phase was transferred into Pyrex glass tubes, and 2.5 ml of a toluene/methanol (1:4 v/v) mixture and 200 µl of acetyl chloride were added. After 1 h in an oven at 100°C, 5 ml of K_2CO_3 (6% w/v in water) was added to the silica. After centrifugation at 1200 g x 10 min, the supernatant was transferred into amber glass vials, dried under nitrogen and resuspended in 100 µl of hexane before gas chromatography analysis.

The gas chromatography analysis was performed as described by Ackman (1986), partly modified. Separations were performed with a 30 m 0.32 mm i.d. Omegawax 320 capillary column, under these conditions: initial isotherm, 140 °C for 5 min; temperature gradient, 2 °C/min to 210°C; final isotherm, 210°C for 20 min. The injector temperature was 250°C. Injection volume was 1 µl with a split ratio of 1/100, and the flame ionization detector temperature was 250°C. Carrier and makeup gas were

hydrogen and nitrogen, respectively. Fatty acid retention times were obtained by injecting the Omegawax test mix as standard.

Statistical analysis

Statistical analysis was carried out by R statistic software (version 3.1.2). One way repeated-measures analysis of variance (ANOVA) was used to compare the data obtained from subjects stratified by sex and by type of hyperlipidemia; post-hoc analysis of differences between paired data was assessed, when appropriate, by the Least Significant Difference. Differences in serum lipid concentrations, anthropometric data and the proportion of FA composition of RBCs in relation to different lipid disorders was evaluated by non-parametric Wilcoxon-Mann-Whitney test with Benjamini-Hochberg correction. The relationship among variables was assessed by Kendall and Spearman rank, non-parametric correlation tests. This approach was used to achieve the significance level and the trend (direct or inverse) of the data correlation. The level of statistical significance was set at $p<0.05$; data are presented as a mean and standard deviation (SD).

Results

Subjects characteristics

The main features of the study sample are summarized in **Table 1**. The cohort of children and adolescents included 54 hyperlipidemic subjects (26 F), aged 6-17 years old; 14 were affected by FH (7 F), 21 by FCHL (12 F) and 19 by PHC (7 F). Five subjects were slightly overweight; mean blood pressure levels were in the normal range.

Serum lipid profile and RBC phospholipid composition

Serum lipid profile and RBC phospholipid composition of the patients, are reported in

Table 2, and classified according to gender and clinical diagnosis. Mean serum lipid parameters exceeded the 90th percentiles (age and sex related), except for HDL-C, showing normal concentrations. RBC phospholipid composition was SFA>PUFA>MUFA, with low omega-3 index value (3.76 ± 1.04%).

No significant serum lipid concentration difference was observed between males and females, while it was detected in the phospholipid composition of RBCs, as females showed higher concentrations of stearic acid but lower DGLA concentration (p= 0.031 and 0.050, respectively). Moreover, MUFA content and MUFA/SFAs ratio in RBCs was lower in females than males (p= 0.052 and 0.056, respectively).

A further analysis considering the type of hyperlipidemia revealed that children with FH had higher levels (p< 0.001) of serum TC, LDL-C and non-HDL-C, and lower serum HDL/LDL ratio if compared to children with FCHL or PHC. Finally, significantly lower levels of vaccenic acid and γ-linolenic acid were detected in FH as compared to PHC children (p= 0.036 and 0.027, respectively).

Correlation between RBC phospholipid composition and serum lipid profile

Correlations between serum lipid levels and RBC phospholipid composition are reported in **Table 3**. Lower RBC MUFA levels correlated with higher serum TC levels (p= 0.032) and HDL/TG ratio (p= 0.025), but lower serum TG levels (p= 0.005). A positive correlation between serum HDL-C and PUFA n-6 levels (p= 0.048) was also detected.

Food diary analysis and correlation between dietary factors, RBC phospholipid FAs and serum lipid profile

Complete and detailed weekly food diaries were obtained from 23 (7 F) out of 54

children, and were used to calculate mean daily energy and macronutrient intake. Results showed a mean daily total energy intake of 1208 ± 151 kcal suggesting a possible overall underestimation of food intake (e.g. in terms of portion size declared). Macronutrients distribution, expressed as percentage of daily energy intake, was: 16.7 ± 2.8% protein, 58.6 ± 6.1% carbohydrate and 24.7 ± 6.4% fat (saturated: 7.4 ± 2.1 %, monounsaturated: 10.3 ± 4.8 %, polyunsaturated: 2.6 ± 0.5 %). Estimated cholesterol intake was 136.2 ± 47.2 mg/day, while fibre intake was 14.0 ± 5.3 g/day, confirming previous data (Guardamagna et al. 2013; Guardamagna et al. 2014). Correlations between dietary factors, the RBC phospholipid composition and serum lipid profile are reported in **Table 4**. An inverse association was found between the dietary intake of cholesterol and n-6 PUFA and n-6 LC-PUFA levels in RBC phospholipids (p= 0.001 and 0.017, respectively). The fibre intake was directly associated with RBC MUFA/SFA ratio (p= 0.035).

Discussion

To our knowledge, this is the first study aimed at characterizing the FA composition of RBC phospholipids in a population of Italian children and adolescents with primary hyperlipidemia.

The phospholipid composition of RBCs has been suggested as an additional marker to monitor serum lipoprotein profile changes (Harris et al. 2004; Novgorodtseva et al. 2001) and as an outcome parameter in the management of hyperlipidemia, although no reference values exist neither for healthy nor hyperlipidemic adults or children. Alterations of the erythrocyte lipid matrix have been demonstrated in studies performed in adults affected by dyslipidemia and/or with risk factors for coronary heart diseases (Laryea et al. 1990; Engelmann et al. 1992; Taylor 1994; Antoku et al. 2000; Lausada et

al. 2007; Vajá et al. 2009; Restic-Medic et al. 2009; Novgorodtseva et al. 2011; Jacobs et al. 2014). For instance, Novgorodtseva et al. (2011) described reduced levels of n-3 PUFA, in particular docosapentaenoic acid (22:5n-3) and DHA (22:6n-3), in dyslipidemic patients as compared to normal subjects. Lausada et al. (2007) documented a PUFA decrease and SFA concentrations increase in plasma and RBC membranes of patients with coronary heart disease, as compared to controls.

Similar studies have been performed in children with diabetes, attention-deficit hyperactive disorder, autism, and other various disorders, except for hyperlipidemia, thus, making the comparison of our data with other data from literature extremely difficult (Decsi et al. 2002; Vanderjagt et al. 2003; Trebbe et al. 2003; Chen et al. 2004; Bu et al. 2006; Vaisman et al. 2008; Burrows et al. 2011; Perona et al. 2013; Brigandi et al. 2015; Bueno et al. 2015). Results obtained in subjects with disorders seem to differ from those obtained in the relative controls. As an example, Perona et al. (2013) found higher levels of SFAs, and reduced concentrations of MUFAs and n-6 PUFAs in RBC membranes of obese adolescents as compared to normal weight controls. These results are in line with our observations as hyperlipidemic children and adolescents showed comparable SFA and MUFA levels. However, it should be also underlined that values obtained in different studies and different countries can be significantly affected by the dietary habits, thus direct comparison of results is not always possible.

It is recognized that children affected by hyperlipidemia exhibit an increased risk of atherosclerosis and CVD during adulthood (McGill et al. 2000; Durrington 2003). Recently, the PANIC study evaluated the association between plasma FA composition (in triacylglycerol and phospholipid fractions), the estimated desaturase/elongase activities and the cardiometabolic risk in 384 children (Venäläinen et al. 2016). Authors found a significant positive correlation between FAs metabolism and the

cardiometabolic risk score for the pediatric population.

The omega-3 index may represent a novel, physiologically relevant and graded risk factor to predict CVD development, thus having a significant clinical utility. A low index (≤4%) was associated with a high risk of mortality for CVD and a value ≥8% was suggested as a reasonable preliminary target for reducing the risk (Harris & Von Schacky 2004; Von Schacky 2010). In the pediatric population, only one other study examined the omega-3 index and concluded that a greater proportion of obese children had a lower index compared with non-obese children (Burrows et al. 2011). In our study, we documented mean omega-3 index <4% in hyperlipidemic children, a factor that contribute to classify them as at future high risk for CVD. Since EPA and DHA derive from essential fatty acids, these results suggest that there is a reduced synthesis or patients did not regularly consume LC-PUFA rich food sources. In this regard, the use of whole dietary strategies and/or specific supplementations (e.g. with PUFA, MUFA or other food bioactives) could be fundamental to improve this index above all in at risk subjects such as hyperlipidemic children.

The present analysis on sex-related differences showed that females had a significantly greater proportion of the SFA stearic acid, and lower proportion of n-6 PUFA, in particular DGLA. Lower DGLA values were also observed in serum glycerophospholipids of healthy young girls when compared with boys (Glaser et al. 2010). Moreover, in females we found a trend towards lower level of MUFA and MUFA/SFA ratio in RBCs with respect to males. Venäläinen et al. (2016) reported that a higher plasma level of SFA myristic acid, MUFA palmitoleic acid and reduced concentrations of n-6 PUFA (linoleic acid) have been correlated with increased cardiometabolic risk in children. Furthermore, higher levels of stearic, palmitic acids and lower levels of linoleic acid in RBC membranes were observed in obese adolescents

(Perona et al. 2013) and in hypercholesterolemic adults (Taylor 1994), as compared to healthy controls. Both sex and gender differences in the pathogenesis, progression and manifestation of atherosclerosis and CVD have been well documented for adults (Maas & Appelman 2010; Mosca et al. 2011; Spence & Pilote 2015), while controversial data have been reported in children (Marelli et al., 2010; Krishnan et al., 2012). In this context, our preliminary findings on sex differences in the pediatric population, associated with higher risk of future coronary diseases, in terms of lipid profile and CVD risks, could be considered by future studies in this field.

Children affected by FH had significantly lower levels of MUFA, vaccenic acid and n-6 PUFA γ-linolenic acid in RBCs, with respect to FCH or PCH patients. These data are consistent with earlier findings in adults demonstrating that subjects with hypercholesterolemia and advanced coronary heart disease exhibited a decreased PUFA level and an increased cholesterol and SFA concentrations in erythrocytes related to the atherosclerotic condition (Taylor 1994; Lausada et al. 2007; Vayá et al. 2009; Novgorodtseva et al. 2011). As far as the pediatric age is concerned, a significantly lower level of vaccenic acid in RBCs was also found in diabetic children, as compared to controls (Decsi et al. 2002).

Correlation analysis performed on the whole study sample (n= 54) showed a positive association between the n-6 PUFA levels in RBCs and serum HDL-C concentrations, which was similar to other observations in young or adults, showing a direct relationship between serum n-6 PUFAs and HDL-C (Ferrucci et al. 2006; Motoyama et al. 2009; Jelenkovic et al. 2014). Furthermore, we observed an inverse correlation between MUFA in RBCs and serum TC concentration and HDL/TG ratio. These results are in agreement with those from previous clinical studies showing that diet rich in MUFA have potential hypocholesterolemic effect (Yu et al. 1995; Gill et al. 2003;

Fernandez et al. 2005; Mukuddem-Peterson et al. 2005). In addition, we found a direct correlation between MUFA in RBCs and serum TG. These results are in line with a study conducted in two cohorts of young healthy twins, in which the contribution of genetic and environmental factors at the bases of relationship between FAs and lipoprotein profile have been performed. Results obtained demonstrated the importance of common genetic factors in determining the phenotypic covariation of n-6 PUFAs and MUFAs with TG and VLDL particles (Jelenkovic et al., 2014). The role of metabolic features and genetic factors on lipid profile is widely studied (Xiang et al. 2007; Matthan et al. 2014; Mayneris-Perxachs et al. 2014; Tosi et al. 2014). For example, recent evidences indicate that FA desaturases play an important role in defining blood and tissue lipid profiles. Polymorphisms in the FA desaturase genes FADS1 and FADS2, that encode respectively for the delta-5 and delta-6 desaturases, have been associated with PUFA levels in both serum phospholipids and RBC membranes (Schaeffer et al. 2006; Malerba et al. 2008; Tosi et al. 2014). Since diet could have also contributed, we tried to correlate dietary factors with lipid profile in both serum and RBCs. A correlation between low intake of dietary cholesterol and high level of n-6 PUFA and n-6 LC-PUFA in RBCs was found. In addition, the fibre intake was directly associated with the MUFA/SFA ratio. These associations have been obtained on a subgroup of children/adolescents involved (23 out of the 54 subjects) and this can represent a possible limitation of the study. However, these results provide additional evidence supporting that a low-cholesterol and fibre-rich diet could contribute to a more favourable RBC phospholipid FA composition in hyperlipidemic children.

Finally, although a limitation of the study is the absence of a normolipidemic control group, our data provides for the first time information about the phospholipid composition of RBCs in a relatively large population of Italian hyperlipidemic children

and adolescents.

Conclusion

In conclusion, although preliminary, we provided experimental evidence about differences in the phospholipid composition of RBCs according to patient type of hyperlipidemia and sex. The low omega-3 index, evaluated on phospholipid RBCs, observed in these children confirms the importance of the quality of dietary FAs intake, particularly in those with primary hyperlipidemia. Further studies should be performed in order to clarify the RBC phospholipid composition differences between hyperlipidemic and normolipidemic children, as well as in relation to sex and diagnosis. Moreover, it would be interesting to study the contribution of specific dietary interventions/regimen on these parameters in children affected by primary hyperlipidemia.

Acknowledgments

We are grateful to Mr Daniele Contino for the support in fatty acid analyses.

Disclosure statement

The authors declare no conflicts of interest regarding this research.

References

Ackman RG. 1986. Wcot (capillary) gas-liquid chromatography. In: Hamilton RJ, Rossel JB, editors. Analysis of oils and fats. London: Elsevier Applied Science Publishers; p. 137–206.

Ahmed SM, Clasen ME, Donnelly JE. 1998. Management of dyslipidemia in adults. Am Fam Physician 57:2192-2204.

Antoku Y, Tsukamoto K, Miyoshi Y, Nagino H, Anezaki M, Suwa K, Narabe Y. 2000. Correlations of elevated levels of hexacosanoate in erythrocyte membranes with risk factors for atherosclerosis. Atherosclerosis 153:169-173.

Brigandi SA, Shao H, Qian SY, Shen Y, Wu BL, Kang JX. 2015. Autistic Children Exhibit Decreased Levels of Essential Fatty Acids in Red Blood Cells. Int J Mol Sci 16:10061-10076.

Bu B, Ashwood P, Harvey D, King IB, Van de Water J, Jin LW. 2006. Fatty acid compositions of red blood cell phospholipids in children with autism. Prostag Leukotr Ess 74:215-221.

Bueno AA, Brand A, Neville MM, Lehane C, Brierley N, Crawford MA. 2015. Erythrocyte phospholipid molecular species and fatty acids of Down syndrome children compared with non-affected siblings. Brit J Nutr 113:72-81.

Burrows T, Collins CE, Garg ML. 2011. Omega-3 index, obesity and insulin resistance in children. Int J Pediatr Obes 6:532-539.

Carnovale E, Marletta L. 2000. Tabelle di composizione degli alimenti: aggiornamento 2000. Istituto Nazionale di Ricerca per gli Alimenti e la Nutrizione. Milano: Edizioni Edra.

Catapano AL, Reiner Ž, De Backer G, Graham I, Taskinen MR, Wiklund O, Agewall S, Alegria E, Chapman M, Durrington P, Erdine S, Halcox J, Hobbs R, Kjekshus J,

Filardi PP, Riccardi G, Storey RF, Wood D, European Society of Cardiology (ESC), European Atherosclerosis Society (EAS). 2011. ESC/EAS Guidelines for the management of dyslipidaemias. The Task Force for the management of dyslipidaemias of the European Society of Cardiology (ESC) and the European Atherosclerosis Society (EAS). Atherosclerosis 217:3-46.

Chen JR, Hsu SF, Hsu CD, Hwang LH, Yang SC. 2004. Dietary patterns and blood fatty acid composition in children with attention-deficit hyperactivity disorder in Taiwan. J Nutr Biochem 15:467-472.

Cortés E, Rizo-Baeza MM, Aguilar MJ, Hidalgo MJ, Gil V. 2012. Correspondence between the fatty acids in healthy children serum and in membrane phospholipids. Nutrición Hospitalaria 28:1541-1545.

Decsi T, Minda H, Hermann R, Kozári A, Erhardt E, Burus I, Soltész G. 2002. Polyunsaturated fatty acids in plasma and erythrocyte membrane lipids of diabetic children. Prostag Leukotr Ess 67:203-210.

Durrington P. Dyslipidemia. 2003. Lancet 362:717–731.

Engelmann B, Streich S, Schönthier UM, Richter WO, Duhm J. 1992. Changes of membrane phospholipid composition of human erythrocytes in hyperlipidemias. I. Increased phosphatidylcholine and reduced sphingomyelin in patients with elevated levels of triacylglycerol-rich lipoproteins. BBA-Lipid Lipid Met 1165:32-37.

Expert panel on integrated guidelines for cardiovascular health and risk reduction in children and adolescents: summary report. Pediatrics 2011; 128(S5):S213-S256.

Farquhar JW, Ahrens Jr EH. 1963. Effects of dietary fats on human erythrocyte fatty acid patterns. J Clin Invest 42:675–685.

Fernandez ML, West KL. 2005. Mechanisms by which dietary fatty acids modulate plasma lipids. J Nutr 135:2075-2078.

Ferrucci L, Cherubini A, Bandinelli S, Bartali B, Corsi A, Lauretani F, Martin A, Andres-Lacueva C, Senin U, Guralnik JM. 2006. Relationship of plasma polyunsaturated fatty acids to circulating inflammatory markers. J Clin Endocrinol Metab 91:439–446.

Gill JMR, Brown JC, Caslake MJ, Wright DM, Cooney J, Bedford, D, Hughes DA, Stanley JC, Packard CJ. 2003. Effects of dietary monounsaturated fatty acids on lipoprotein concentrations, compositions, and subfraction distributions and on VLDL apolipoprotein B kinetics: dose-dependent effects on LDL. Am J Clin Nutr 78:47-56.

Glaser C, Demmelmair H, Sausenthaler S, Herbarth O, Heinrich J, Koletzko B. 2010. Fatty acid composition of serum glycerophospholipids in children. J Pediatr 157:826-831.

Groner JA, Joshi M, Bauer JA. 2006. Pediatric precursors of adult cardiovascular disease: noninvasive assessment of early vascular changes in children and adolescents. Pediatrics 118:1683–1691.

Guardamagna O, Abello F, Anfossi G, Pirro M. 2011. Lipoprotein(a) and family history of cardiovascular disease in children with familial dyslipidemias. J Pediatr 159:314–319.

Guardamagna O, Abello F, Cagliero P, Visioli F. 2013. Could dyslipidemic children benefit from glucomannan intake? Nutrition 29:1060-1065.

Guardamagna O, Amaretti A, Puddu PE, Raimondi S, Abello F, Cagliero P, Rossi M. 2014. Bifidobacteria supplementation: effects on plasma lipid profiles in dyslipidemic children. Nutrition 30:831.

Haney EM, Huffman LH, Bougatsos, C, Freeman M, Steiner RD, Helfand M, Nelson HD. 2007. Screening and treatment for lipid disorders in children and adolescents: systematic evidence review for the US Preventive Services Task Force. Pediatrics 120:189-214.

Harris WS, Sands SA, Windsor SL, Ali HA, Stevens TL, Magalski A, Porter CB, Borkon AM. 2004. Omega-3 fatty acids in cardiac biopsies from heart transplantation patients correlation with erythrocytes and response to supplementation. Circulation 110:1645-1649.

Harris WS, Thomas RM. 2010. Biological variability of blood omega-3 biomarkers. Clinical biochemistry 43:338-340.

Harris WS, Von Schacky C. 2004. The Omega-3 Index: a new risk factor for death from coronary heart disease?. Prev Med 39:212-220.

Hodson L, Skeaff CM, Fielding BA. 2008. Fatty acid composition of adipose tissue and blood in humans and its use as a biomarker of dietary intake. Progress Lip Res 47:348-380.

Jacobs S, Schiller K, Jansen E, Fritsche A, Weikert C, di Giuseppe R, Boeing H, Schulze MB, Kröger J.2014. Association between erythrocyte membrane fatty acids and biomarkers of dyslipidemia in the EPIC-Potsdam study. Eur J Clin Nutr 68:517-525.

Jakobik V, Burus I, Decsi T. 2009. Fatty acid composition of erythrocyte membrane lipids in healthy subjects from birth to young adulthood. Eur J Pediatr 168:141-147.

Jelenkovic A, Bogl LH, Rose RJ, Kangas AJ, Soininen P, Ala-Korpela M, Kaprio J, Silventoinen K. 2014. Association between serum fatty acids and lipoprotein

subclass profile in healthy young adults: Exploring common genetic and environmental factors. Atherosclerosis 233:394-402.

Krishnan S, Fields DA, Copeland KC, Blackett PR, Anderson MP, Gardner AW. 2012. Gender Differences in Cardiovascular Risk in Adolescents with Type 1 Diabetes. Gender Med 9:251–258.

Kuratko, CN, Salem N. 2009. Biomarkers of DHA status. Prostag Leukotr Ess 81:111-118.

Laryea M, Cieslicki P, Diekmann E, Wendel U. 1990. Age-dependent fatty acid composition of erythrocyte membrane phospholipids in healthy children. Z Ernahrungswiss 1990;29:284-294.

Lausada NR, Boullon S, Boullon F, de Gomez Dumm INT. 2007. Erythrocyte membrane, plasma and atherosclerotic plaque lipid pattern in coronary heart disease. Medicina-B. Aires 67:451.

Maas AHEM, Appelman YEA. 2010. Gender differences in coronary heart disease. Neth Heart J 18:598-603.

Malerba G, Schaeffer L, Xumerle L, Klopp N, Trabetti E, Biscuola M, Cavallari U, Galavotti R, Martinelli N, Guarini P, Girelli D, Olivieri O, Corrocher R, Heinrich J, Pignatti PF, Illig T. 2008. SNPs of the FADS gene cluster are associated with polyunsaturated fatty acids in a cohort of patients with cardiovascular disease. Lipids 43:289–99.

Marelli A, Gauvreau K, Landzberg M, Jenkins K. 2010. Sex differences in mortality in children undergoing congenital heart disease surgery a united states population–based study. Circulation122:S234-S240.

Marks PA, Gellhorn A, Kidson C. 1960. Lipid synthesis in human leukocytes, platelets, and erythrocytes. J Biol Chem 235:2579–2583.

Matthan NR, Ooi EM, Van Horn L, Neuhouser ML, Woodman R, Lichtenstein AH. 2014. Plasma phospholipid fatty acid biomarkers of dietary fat quality and endogenous metabolism predict coronary heart disease risk: a nested case-control study within the Women's Health Initiative observational study. J Am Heart Assoc 3:e000764.

Mayneris-Perxachs J, Guerendiain M, Castellote AI, Estruch R, Covas MI, Fitó M, Salas-Salvadó J, Martínez-González MA, Aros F, Lamuela-Raventós RM, López-Sabater MC. 2014. Plasma fatty acid composition, estimated desaturase activities, and their relation with the metabolic syndrome in a population at high risk of cardiovascular disease. Clinical nutrition, 33:90-97.

McGill Jr HC, McMahan CA, Herderick EE, Malcom GT, Tracy RE, Strong JP. 2000. Origin of atherosclerosis in childhood and adolescence. Am J Clin Nutr 72:1307s-1315s.

Mosca L, Barrett-Connor E, Wenger NK. 2011. Sex/gender differences in cardiovascular disease prevention what a difference a decade makes. Circulation 124:2145-2154.

Motoyama KR, Curb JD, Kadowaki T, El-Saed A, Abbott RD, Okamura T, Evans RW, Nakamura Y, Sutton-Tyrrell K, Rodriquez BL, Kadota A, Edmundowicz D, Willcox BJ, Choo J, Katsumi N, Otake T, Kadowaki S, Kuller LH, Ueshima H, Sekikawa A. 2009. Association of serum n-6 and n-3 polyunsaturated fatty acids with lipids in 3 populations of middle-aged men. Am J Clin Nutr 90:49–55.

Mukuddem-Petersen J, Oosthuizen W, Jerling JC. 2005. A systematic review of the effects of nuts on blood lipid profiles in humans. J Nutr 135:2082-2089.

Newman WP 3rd, Freedman DS, Voors AW, Gard PD, Srinivasan SR, Cresanta JL, Williamson GD, Webber LS, Berenson GS. 1986. Relation of serum lipoprotein

levels and systolic blood pressure to early atherosclerosis: the Bogalusa Heart Study. N Engl J Med 314:138–144.

Novgorodtseva TP, Kantur TA, Karaman YK, Antonyuk MV, Zhukova NV. 2011. Modification of fatty acids composition in erythrocytes lipids in arterial hypertension associated with dyslipidemia. Lipids Health Dis 10:18.

Perona JS, González-Jiménez E, Aguilar-Cordero MJ, Sureda A, Barceló F. 2013. Structural and compositional changes in erythrocyte membrane of obese compared to normal-weight adolescents. J Membrane Biol 246:939-947.

Reed CF. 1968. Phospholipid exchange between plasma and erythrocytes in man and the dog. J Clin Invest 47:749–760.

Rice HB, Bernasconi A, Maki K C, Harris WS, von Schacky C, Calder PC. 2016. Conducting omega-3 clinical trials with cardiovascular outcomes: Proceedings of a workshop held at ISSFAL 2014. Prostaglandins Leukot Essent Fatty Acids 107:30-42.

Ristic-Medic D, Suzic S, Vucic V, Takic M, Tepsic J, Glibetic M. 2009. Serum and erythrocyte membrane phospholipids fatty acid composition in hyperlipidemia: effects of dietary intervention and combined diet and fibrate therapy. Gen Physiol Biophys 28:190-199.

Sarkkinen, ES, Agren JJ, Ahola I, Ovaskainen ML, Uusitupa MI. 1994. Fatty acid composition of serum cholesterol esters, and erythrocyte and platelet membranes as indicators of long-term adherence to fat-modified diets. Am J Clin Nutr 59:364-370.

Schaeffer L, Gohlke H, Müller M, Heid IM, Palmer LJ, Kompauer I, Demmelmair H, Illig T, Koletzko B, Heinrich J. 2006. Common genetic variants of the FADS1

FADS2 gene cluster and their reconstructed haplotypes are associated with the fatty acid composition in phospholipids. Hum Mol Genet 15:1745–56.

Simonetti P, Ciappellano S, Gardana C, Bramati L, Pietta P. 2002. Procyanidins from Vitis vinifera seeds: in vivo effects on oxidative stress. J Agr Food Chem 50:6217-6221.

Spence JD, Pilote L. 2015. Importance of sex and gender in atherosclerosis and cardiovascular disease. Atherosclerosis 241:208-210.

Taylor AJ. 1994. Erythrocyte membrane fatty acid composition in hypercholesterolemia. Ann Clin Biochem 31:351-354.

Tosi F, Sartori F, Guarini P, Olivieri O, Martinelli N. 2014. Delta-5 and Delta-6 Desaturases: Crucial Enzymes in Polyunsaturated Fatty Acid-Related Pathways with Pleiotropic Influences in Health and Disease. In: Camps J, editor. Oxidative Stress and Inflammation in Non-communicable Diseases - Molecular Mechanisms and Perspectives in Therapeutics. Advances in Experimental Medicine and Biology 824. Cham: Springer International Publishing; p. 61–81.

Trebble TM, Wootton SA, May A, Erlewyn-Lajeunesse MDS, Chakraborty A, Mullee MA, Stroud MA, Beattie RM. 2003. Essential fatty acid status in paediatric Crohn's disease: relationship with disease activity and nutritional status. Aliment Pharm Therap 18:433-442.

Tziakas DN, Chalikias GK, Stakos D, Boudoulas H. 2010. The role of red blood cells in the progression and instability of atherosclerotic plaque. Int J Cardiol 142:2-7.

Vaisman N, Kaysar N, Zaruk-Adasha Y, Pelled D, Brichon G, Zwingelstein G, Bodennec J. 2008. Correlation between changes in blood fatty acid composition and visual sustained attention performance in children with inattention: effect of dietary n−3 fatty acids containing phospholipids. Am J Clin Nutr 87:1170-1180.

VanderJagt DJ, Trujillo MR, Bode-Thomas F, Huang YS, Chuang LT, Glew RH. 2003. Phase angle correlates with n-3 fatty acids and cholesterol in red cells of Nigerian children with sickle cell disease. Lipids Health Dis 2:1-8.

Vayá A, Martínez Triguero M, Réganon E, Vila V, Martínez Sales V, Solá E, Hernández Mijares A, Ricart A. 2009. Erythrocyte membrane composition in patients with primary hypercholesterolemia. Clin Hemorheol Micro 41:67.

Venäläinen T, Ågren J, Schwab U, de Mello VD, Eloranta AM, Laaksonen DE, Lindi V, Lakka TA. 2016. Cross-sectional associations of plasma fatty acid composition and estimated desaturase and elongase activities with cardiometabolic risk in Finnish children—The PANIC study. J Clin Lipidol 10:82–91.

Von Schacky C. 2010. Omega-3 index and sudden cardiac death. Nutrients 2;375-388.

Xiang M, Rahman MA, Ai H, Li X, Harbige LS. 2007. Diet and gene expression: delta-5 and delta-6 desaturases in healthy Chinese and European subjects. Ann Nutr Metab 50:492-498.

Yu S, Derr J, Etherton TD, Kris-Etherton PM. 1995. Plasma cholesterol-predictive equations demonstrate that stearic acid is neutral and monounsaturated fatty acids are hypocholesterolemic. Am J Clin Nutr 61:1129-1139.

Table 1. Subject characteristics (n= 54)

Parameter	Value
Age, years (range)	11 ± 3 (6-17)
Sex, M/F	28/26
Weight, kg	47.9 ± 16.4
Height, cm	149.1 ± 16.1
BMI, kg/m^2	20.9 ± 4.0
Systolic blood pressure, mmHg	104.5 ± 10.1
Diastolic blood pressure, mmHg	66.6 ± 7.4

Notes: BMI, body mass index. Values are reported as mean ± SD.

Table 2. Serum lipid concentrations and RBC phospholipid FAs composition in hyperlipidemic children and adolescents (n= 54), according to sex and type of hyperlipidemia

Lipid profile	All subjects	Sex			Dyslipidemia			
	n= 54	Male n= 28	Female n= 26	*p*-value	FH n=14	FCHL n= 21	PHC n= 19	*p*-value
Serum lipids (mg/dl)								
TC	232.4 ± 55.6	226.0 ± 56.7	239.2 ± 54.8	0.385	288.2 ± 67.1[a]	214.2 ± 38.3[b]	211.4 ± 30.8[b]	< 0.001
TG	90.9 ± 51.9	94.0 ± 53.4	84.9 ± 50.3	0.658	73.6 ± 31.3	102.3 ± 67.6	91.1 ± 42.1	0.282
HDL-C	58.8 ± 15.4	58.6 ± 15.6	60.2 ± 14.6	0.912	55.0 ± 10.6	57.8 ± 19.6	62.7 ± 12.7	0.340
LDL-C	156.3 ± 58.4	149.1 ± 60.9	163.3 ± 56.8	0.355	219.9 ± 69.5[a]	137.3 ± 36.5[b]	130.3 ± 28.0[b]	< 0.001
HDL/LDL ratio	0.4 ± 0.2	0.4 ± 0.2	0.4 ± 0.2	0.502	0.3 ± 0.1[a]	0.5 ± 0.2[b]	0.5 ± 0.2[b]	< 0.001
HDL/TG ratio	0.9 ± 0.5	0.8 ± 0.5	0.9 ± 0.5	0.537	0.9 ± 0.5	0.8 ± 0.5	0.8 ± 0.4	0.812
Non-HDL-C	173.6 ± 59.4	167.4 ± 61.7	179.0 ± 58.1	0.433	233.2 ± 71.0[a]	156.5 ± 42.5[b]	148.6 ± 31.4[b]	< 0.001
RBC phospholipid FAs (%)								
Total SFAs	48.99 ± 2.23	48.66 ± 2.51	49.44 ± 1.84	0.261	49.23 ± 2.87	49.35 ± 1.53	48.43 ± 2.35	0.389
Total MUFAs	18.45 ± 0.91	18.68 ± 0.84	18.12 ± 0.87	0.052	18.07 ± 0.77	18.59 ± 0.86	18.58 ± 1.02	0.197
Total PUFAs	32.55 ± 2.14	32.65 ± 2.55	32.44 ± 1.67	0.729	32.70 ± 2.81	32.06 ± 1.61	32.99 ± 2.10	0.383
MUFAs/SFAs ratio	0.38 ± 0.03	0.39 ± 0.03	0.37 ± 0.03	0.056	0.37 ± 0.03	0.38 ± 0.02	0.39 ± 0.03	0.296
PUFAs/SFAs ratio	0.67 ± 0.08	0.68 ± 0.09	0.66 ± 0.06	0.452	0.67 ± 0.11	0.65 ± 0.05	0.68 ± 0.08	0.391
PUFAs n-3/n-6	0.19 ± 0.06	0.18 ± 0.03	0.20 ± 0.05	0.758	0.19 ± 0.05	0.20 ± 0.07	0.19 ± 0.04	0.871
LC-PUFAs n-3/n-6	0.33 ± 0.09	0.31 ± 0.05	0.34 ± 0.09	0.633	0.33 ± 0.09	0.34 ± 0.11	0.32 ± 0.08	0.822
Omega-3 index	3.76 ± 1.04	3.54 ± 0.55	3.80 ± 1.08	0.770	3.81 ± 0.87	3.84 ± 1.24	3.62 ± 0.95	0.789
Saturated								
14:0 (myristic acid)	0.41 ± 0.08	0.40 ± 0.09	0.42 ± 0.08	0.336	0.42 ± 0.10	0.4 ± 0.07	0.4 ± 0.08	0.789
15:0 (pentadecanoic acid)	0.16 ± 0.03	0.16 ± 0.03	0.17 ± 0.03	0.152	0.16 ± 0.03	0.16 ± 0.02	0.16 ± 0.03	0.963

16:0 (palmitic acid)	24.05 ± 1.38	24.02 ± 1.52	24.08 ± 1.23	0.864	24.4 ± 1.56	24.06 ± 1.10	23.78 ± 1.52	0.625
17:0 (margaric acid)	0.46 ± 0.17	0.46 ± 0.16	0.45 ± 0.19	0.803	0.42 ± 0.22	0.44 ± 0.19	0.51 ± 0.11	0.387
18:0 (stearic acid)	15.47 ± 1.47	15.06 ± 1.40[a]	15.92 ± 1.44[b]	0.031	15.61 ± 2.10	15.68 ± 1.16	15.15 ± 1.24	0.593
20:0 (arachidic acid)	0.56 ± 0.15	0.58 ± 0.20	0.55 ± 0.05	0.579	0.54 ± 0.07	0.55 ± 0.05	0.6 ± 0.23	0.610
22:0 (behenic acid)	1.99 ± 0.28	1.98 ± 1.29	1.99 ± 1.27	0.988	1.94 ± 0.32	2.02 ± 0.24	1.98 ± 0.29	0.607
23:0 (tricosanoic acid)	0.31 ± 0.06	0.31 ± 0.05	0.31 ± 0.06	0.935	0.30 ± 0.07	0.32 ± 0.05	0.31 ± 0.05	0.576
24:0 (lignoceric acid)	5.59 ± 0.75	5.7 ± 0.84	5.46 ± 0.63	0.242	5.45 ± 0.83	5.73 ± 0.63	5.54 ± 0.81	0.583
Monounsaturated								
16:1n-9 (hypogeic acid)	0.11 ± 0.03	0.11 ± 0.01	0.11 ± 0.04	0.524	0.11 ± 0.04	0.11 ± 0.03	0.11 ± 0.01	0.934
16:1n-7 (palmitoleic acid)	0.24 ± 0.07	0.24 ± 0.07	0.23 ± 0.07	0.561	0.23 ± 0.08	0.23 ± 0.08	0.25 ± 0.05	0.795
18:1n-9 (oleic acid)	11.09 ± 0.94	11.16 ± 0.89	11.02 ± 1.01	0.569	11.19 ± 1.16	10.95 ± 0.91	11.18 ± 0.83	0.719
18:1n-7 (vaccenic acid)	1.08 ± 0.11	1.07 ± 0.10	1.09 ± 0.12	0.392	1.02 ± 0.07[a]	1.08 ± 0.11[ab]	1.12 ± 0.10[b]	0.036
20:1n-9 (eicosenoic acid)	0.20 ± 0.08	0.21 ± 0.10	0.19 ± 0.02	0.474	0.18 ± 0.02	0.19 ± 0.02	0.22 ± 0.12	0.361
24:1n-9 (nervonic acid)	5.73 ± 0.92	5.9 ± 1.14	5.56 ± 0.56	0.179	5.34 ± 1.11	6.02 ± 0.60	5.70 ± 0.99	0.240
n-6 Polyunsaturated								
18:2n-6 (linoleic acid)	10.70 ± 1.08	10.72 ± 1.06	10.68 ± 1.13	0.916	10.49 ± 0.77	10.78 ± 1.13	10.78 ± 1.24	0.795
18:3n-6 (-linolenic acid)	0.06 ± 0.02	0.06 ± 0.02	0.06 ± 0.02	0.193	0.06 ± 0.02[a]	0.06 ± 0.02[a]	0.07 ± 0.02[b]	0.027
20:2n-6 (eicosadienoic acid)	0.23 ± 0.07	0.24 ± 0.09	0.22 ± 0.05	0.222	0.20 ± 0.06	0.23 ± 0.03	0.26 ± 0.10	0.138
20:3n-6 (dihomo-γ-linolenic acid)	1.91 ± 0.38	2.01 ± 0.46[a]	1.81 ± 0.24[b]	0.050	1.75 ± 0.26	1.95 ± 0.37	1.99 ± 0.44	0.137
20:4n-6 (arachidonic acid)	11.05 ± 1.14	10.89 ± 1.29	11.23 ± 0.95	0.270	11.43 ± 1.23	10.84 ± 0.86	11.01 ± 1.33	0.446
22:4n-6 (adrenic acid)	1.93 ± 0.49	1.92 ± 0.53	1.94 ± 0.44	0.867	1.9 ± 0.53	1.76 ± 0.27	2.14 ± 0.58	0.114
Total PUFA n-6	25.89 ± 1.58	26.15 ± 1.37	25.85 ± 1.50	0.815	25.83 ± 1.69	25.62 ± 1.58	26.25 ± 1.51	0.457
Total LC-PUFAs n-6	14.90 ± 1.46	15.07 ± 1.19	14.82 ± 1.64	0.681	15.08 ± 1.65	14.56 ± 1.16	15.14 ± 1.59	0.392
n-3 Polyunsaturated								
18:3n-3 (α-linolenic acid)	0.09 ± 0.04	0.09 ± 0.02	0.09 ± 0.02	0.827	0.09 ± 0.04	0.09 ± 0.04	0.10 ± 0.03	0.291
20:5n-3 (eicosapentaenoic acid)	0.36 ± 0.21	0.38 ± 0.21	0.33 ± 0.22	0.402	0.37 ± 0.29	0.35 ± 0.22	0.36 ± 0.12	0.988

22:5n-3 (docosapentaenoic acid)	1.10 ± 0.20	1.13 ± 0.21	1.07 ± 0.17	0.207	1.08 ± 0.20	1.04 ± 0.19	1.18 ± 0.19	0.130
22:6n-3 (docosahexaenoic acid)	3.40 ± 0.89	3.41 ± 0.96	3.38 ± 0.82	0.888	3.43 ± 0.67	3.49 ± 1.05	3.26 ± 0.86	0.781
Total PUFAs n-3	4.95 ± 1.17	4.68 ± 0.63	5.02 ± 1.21	0.632	4.98 ± 0.99	4.97 ± 1.39	4.90 ± 1.06	0.976
Total LC-PUFAs n-3	4.86 ± 1.17	4.59 ± 0.63	4.93 ± 1.21	0.637	4.89 ± 0.99	4.88 ± 1.39	4.80 ± 1.06	0.970

Notes: FH, familiar hypercholesterolemia; FCHL, familiar combined hyperlipidemia; PHC, polygenic hypercholesterolemia; TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; LC-PUFAs, long chain polyunsaturated fatty acids (C ≥20, double bonds ≥ 3); omega-3 index, sum of EPA + DHA. Values are reported as mean ± SD. [a,b] Data with different letters are significantly different (p<0.05).

Table 3. Correlation between RBC phospholipid FAs and serum lipid concentrations (n= 54)

RBC phospholipid fatty acids	Serum Lipids					
	TC	HDL-C	LDL-C	TG	HDL/LDL	HDL/TG
Total SFAs	0.095	-0.001	0.095	-0.077	-0.051	0.022
Total MUFAs	-0.290*	-0.138	-0.274	0.371*	0.064	-0.308*
Total PUFAs	0.020	0.072	0.021	-0.137	0.030	0.161
MUFAs/SFAs ratio	-0.208	-0.057	-0.210	0.262	0.073	-0.180
PUFAs/SFAs ratio	-0.025	0.029	-0.015	-0.058	0.021	0.084
Total PUFAs n-3	0.104	-0.155	0.151	-0.068	-0.200	0.011
Total PUFAs n-6	0.023	0.275*	-0.028	-0.155	0.198	0.250
PUFAs n-3/n-6	0.085	-0.220	0.151	-0.039	-0.231	-0.043
Total LC-PUFAs n-3	0.105	-0.168	0.159	-0.064	-0.213	0.000
Total LC-PUFAs n-6	0.174	0.207	0.120	-0.197	0.063	0.243
LC-PUFAs n-3/n-6	-0.020	-0.229	0.059	0.006	-0.181	-0.080
Omega-3 index	0.085	-0.203	0.160	-0.080	-0.233	-0.006

Notes: TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SFAs, saturated fatty acids; MUFAs monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; LC-PUFAs, long chain polyunsaturated fatty acids; omega-3 index, sum of EPA + DHA. *Statistical significance after Kendall and Spearman correlations for multiple comparisons (p<0.05).

Table 4. Correlation between dietary factors, RBC phospholipid FAs and serum lipids (n= 23)

	Dietary factors										
	Energy	Protein	Carbohydrate	Total Fat	SFAs	MUFAs	PUFAs	PUFAs n-3	PUFAs n-6	Cholesterol	Fiber
Serum lipids											
TC	-0.265	-0.202	0.302	-0.204	-0.196	-0.221	-0.376	0.011	-0.083	-0.386	-0.210
HDL-C	-0.381	-0.126	0.163	-0.149	0.118	-0.102	-0.034	-0.087	-0.182	-0.254	-0.203
TG	-0.013	-0.101	-0.210	0.249	0.402	0.123	0.181	-0.007	0.099	0.180	0.104
LDL-C	-0.219	-0.243	0.187	-0.079	-0.147	-0.118	-0.339	0.204	-0.027	-0.280	-0.214
HDL/LDL ratio	-0.252	0.012	-0.012	-0.049	0.167	0.017	0.147	-0.081	-0.113	-0.061	-0.074
HDL/TG ratio	-0.289	-0.054	0.282	-0.289	-0.194	-0.147	-0.154	-0.010	-0.145	-0.277	-0.196
RBC phospholipid FAs											
Total SFAs	-0.145	-0.108	0.150	-0.096	-0.297	-0.105	-0.010	-0.350	0.083	0.265	-0.235
Total MUFAs	0.103	-0.137	-0.142	0.123	0.547	0.292	0.409	0.265	0.282	0.360	0.493
Total PUFAs	-0.059	0.174	-0.044	0.049	0.081	-0.047	-0.203	0.277	-0.216	-0.485	-0.012
MUFAs/SFAs ratio	0.193	-0.056	-0.150	0.123	0.504	0.271	0.324	0.340	0.212	0.088	0.525*
PUFAs/SFAs ratio	0.072	0.146	-0.093	0.069	0.164	0.027	-0.108	0.321	-0.128	-0.407	0.131
Total PUFAs n-3	0.360	0.434	-0.397	0.181	-0.039	0.027	0.017	0.105	0.039	0.137	0.135
Total PUFAs n-6	-0.164	-0.105	0.115	0.029	-0.076	-0.054	-0.282	0.201	-0.243	-0.762*	-0.245
PUFAs n-3/n-6	0.363	0.429	-0.409	0.167	-0.059	0.010	0.029	0.078	0.049	0.260	0.150
Total LC-PUFAs n-3	0.360	0.434	-0.397	0.181	-0.039	0.027	0.017	0.105	0.039	0.137	0.135
Total LC-PUFAs n-6	0.002	0.071	-0.201	0.118	0.108	0.051	-0.108	0.225	-0.157	-0.522*	-0.142
LC-PUFAs n-3/n-6	0.368	0.463	-0.390	0.135	-0.083	-0.034	0.007	0.020	0.034	0.390	0.176
Omega-3 index	0.358	0.441	-0.350	0.127	-0.076	-0.032	-0.010	0.081	0.029	0.265	0.154

Notes: TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SFAs, saturated fatty acids; MUFAs monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; LC-PUFAs, long chain polyunsaturated fatty acids; omega-3 index, sum of EPA + DHA. *Statistical significance after Kendall and Spearman correlations for multiple comparisons (p<0.05).